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Plasma proteins and coronary atherosclerosis
A Mendelian randomization study

Henan Pan, MM[a,b], Zongkai Wu, MD[b], Yaran Gao, MD[b], Wentao Yao, MM[a,b], Ge Feng, MM[a,b], Hebo Wang, PhD[b,c,*]

Abstract

Coronary atherosclerosis (AS) is a complicated and severe chronic pathological process that contributes to the basis of various cardiovascular diseases, which causes a serious challenge to the global healthcare system. AS is the underlying physiopathological mechanism. Despite recent advances in the research of biomarkers and therapeutic targets for AS, there remain significant limitations in the current targeted therapies for AS. This study utilizes Mendelian randomization analysis to leverage genetic variations in order to identify plasma proteins with causal relationships to coronary AS. Utilizing publicly available genome-wide association study datasets, 4907 plasma proteins were assessed as exposure factors, with coronary AS being the outcome variable. The primary analytical method employed was the inverse variance weighted approach to ensure the robustness and accuracy of the causal relationships. In addition, to verify the reliability of the results, we employed several complementary methods, including the weighted median, Mendelian randomization-Egger, weighted mode, and simple mode approaches, to thoroughly assess the heterogeneity and pleiotropy of the findings. To ensure the robustness of the results and to exclude potential biases, a leave-one-out sensitivity analysis was performed. Twenty potential therapeutic targets were analyzed and identified ($P < .05$), combined with multiple bioinformatic analyses; among them, fibronectin 1 was identified as a key target. These findings may provide a new theoretical basis for future research in coronary AS drug development and therapeutic strategies.

Abbreviations: AS = atherosclerosis, CAD = coronary artery disease, FN1 = fibronectin 1, GO = Gene Ontology, GWAS = genome-wide association study, IV = instrumental variable, IVW = inverse variance weighted, KEGG = Kyoto Encyclopedia of Genes and Genomes, MR = Mendelian randomization, PPI = protein-protein interaction, SNP = single-nucleotide polymorphism.

Keywords: atherosclerosis, coronary atherosclerosis, Mendelian randomization, plasma proteins

1. Introduction

Coronary atherosclerosis (AS) is a life-threatening disorder and a significant global health issue. Its primary mechanism, AS,[1] involves damage to endothelial cells and lipid deposition, resulting in arterial thickening, hardening, and plaque formation, which can give rise to severe complications.[2] According to the World Health Organization, cardiovascular diseases cause ≈17.9 million deaths every year, accounting for 32% of global mortality.[3] Despite the existing treatments, the complex etiology of AS requires further exploration to identify novel therapeutic targets and biomarkers, providing new strategies for the prevention and management of cardiovascular diseases.[4]

Plasma proteomics and Mendelian randomization (MR) are essential methodologies for the identification of biomarkers and the exploration of disease mechanisms. Proteins, as primary executors of biological functions, play critical roles in interorgan communication, biological regulation, and homeostasis.[5] Recent advancements in proteomic technologies now facilitate a detailed analysis of proteins, thereby enabling the identification of disease-specific plasma proteins. Numerous studies have established connections between plasma proteins and AS. For instance, Stakhneva et al[6] identified associations between coronary plaque instability and plasma concentrations of complement components, hemopexin, and haptoglobin. Similarly, Stakhneva et al[7] reported differential expression patterns of functional proteins such as C9, C3, and transthyretin in patients with AS. Despite these advancements, traditional clinical studies encounter limitations when it comes to identifying reliable biomarkers for AS. The integration of genome-wide association studies (GWASs) with proteomics offers deeper insights into the

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[a] Department of Graduate School, Hebei Medical University, Shijiazhuang, Hebei, China, [b] Neurology Department, Hebei General Hospital, Shijiazhuang, Hebei, China, [c] Hebei Provincial Key Laboratory of Cerebral Networks and Cognitive Disorders, Shijiazhuang, Hebei, China.

** Correspondence: Hebo Wang, Department of Graduate School, Hebei Medical University, No. 361 Zhongshan Road East, Yucai Subdistrict, Shijiazhuang, Hebei 050000, China or Neurology Department, Hebei General Hospital, No. 348 Heping West Road, Shijiazhuang, Hebei 050051, China (e-mail: wanghbhope@hebmu.edu.cn).*

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genetic regulation of protein levels and their causal relationships with AS, thereby advancing biomarker discovery efforts significantly.

MR is a method that uses genetic variations as instrumental variables (IVs) to evaluate the causal relationship between an exposure and an outcome. This method is based on Mendel laws of inheritance, utilizing the random distribution of genetic variations during meiosis to reduce confounding factors and reverse causality in the assessment of causal relationships.[8] For example, Ren et al[9] assessed the causal relationship between nonalcoholic fatty liver disease (exposure) and coronary artery disease (CAD; outcome) through MR analysis, and a study by Li et al[10] explored the potential causal relationship between aspirin use (exposure) and erectile dysfunction (outcome) through MR analysis. In this study, we performed a systematic, proteome-wide MR analysis to identify exposures and conducted a 2-sample MR analysis using publicly available AS data from GWAS as the outcome. This approach aimed to identify plasma proteins with a causal relationship to coronary AS. In addition, we employed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, as well as constructed a protein-protein interaction (PPI) network, to analyze the functions and pathways of the plasma proteins. We also conducted bioinformatics analysis to evaluate and identify potential key target genes, protein biomarkers, and underlying mechanisms.

2. Materials and methods

2.1. Data source

2.1.1. Exposure data. The plasma protein data acquired using this study are provided from a large-scale data integration of 4907 plasma proteins measured in 35,559 Icelanders using the SomaScan platform through the deCODE Health study by Ferkingstad et al.[11] (PMID: 34857953, https://www.decode.com/summarydata).

2.1.2. Outcome data. We selected coronary AS as the outcome measure and screened for relevant outcome indicators from currently published GWAS data (https://gwas.mrcieu.ac.uk/). The summary data for the outcomes were obtained from the following website: https://gwas.mrcieu.ac.uk/datasets/ukb-d-I9_CORATHER/ (GWSA ID: ukb-d-I9_CORATHER). The study included 361,194 individuals of European ancestry (14,334 cases and 346860 controls) for coronary AS, and a total of 13,586,589 single-nucleotide polymorphisms (SNPs) were analyzed.

All the aforementioned statistical data are available for free download from anonymous public data websites. These datasets have obtained ethical approval, do not require additional informed consent, and can be used without restrictions.[12]

2.2. Selection of IVs

We processed the data to select highly relevant IVs. The specific selection criteria are given as follows.[13] Relevance criteria: ensure that the IVs show a significant association with plasma proteins; specifically, SNPs with a genome-wide significance level ($P < 5 \times 10^{-8}$) exclude SNPs in linkage disequilibrium, using a correlation coefficient threshold of $r^2 < 0.01$ and a clumping distance of 10,000 base pairs. Independence criteria: IVs must be independent of confounding factors (i.e., factors that confound the relationship between plasma proteins and coronary AS). We used PhenoScanner to examine each SNP for potential associations with confounding factors and excluded SNPs that violated the independence assumption. Exclusion criteria: under conditions of exposure and confounding, IV should affect outcomes only through the pathway from the "IV" to the exposure to the outcome. We applied the MR-Egger regression method to calculate the intercept of the fitted model to assess the presence of horizontal pleiotropy. In addition, we used the MR-Steiger test to determine the direction of causality for each SNP, excluding those with incorrect causal directions. SNPs associated with risk factors were used to extract associations in the outcome data and matched, and ambiguous and palindromic SNPs were excluded. For missing SNPs in the outcome data, proxy SNPs with high LD were substituted. Calculate the *F*-statistic for each SNP to assess its strength as an IV, and exclude weak IVs ($F < 10$).[14–16]

2.3. MR analysis

MR helps infer causal relationships between genetic variations and disease outcomes, minimizing the influence of environmental confounding factors. Two-sample MR utilizes data from different samples to reveal protein changes associated with disease and their genetic mechanisms, thereby facilitating the discovery of biomarkers and the development of disease treatment strategies. In this study, plasma proteins were used as exposure variables, and coronary AS was considered the outcome variable. Various methods were applied, including inverse variance weighted (IVW), weighted median, MR-Egger regression, simple mode, and weighted mode, along with several testing approaches. The study comprehensively assessed pleiotropy and heterogeneity in the MR analysis to ensure the accuracy and robustness of causal inferences.[17] When a specific plasma protein has only 1 protein and sequence determinant of protein levels as an IV, the Wald ratio method was used to evaluate its causal effect. When there were ≥2 IVs, the IVW method was applied. Statistical results are expressed as odds ratios and 95% CI with a significance threshold of $P < .05$. The robustness of the results was further validated through 3 specific aspects[18]: testing for genetic pleiotropy, conducting sensitivity analyses, and assessing heterogeneity. First, to assess pleiotropy, we used MR-Egger and MR-PRESSO regression analyses. MR-Egger not only estimates the causal effect between the exposure and the outcome but also detects the presence of horizontal pleiotropy in IVs through the intercept term. The greater the deviation of the intercept term from zero in MR-Egger regression, the higher the likelihood of pleiotropy, which could potentially affect the accuracy of the causal inference. In addition, the MR-PRESSO method can detect outliers and provide causal estimates after removing these outliers to control for and/or test horizontal pleiotropy,[19,20] and corrected adjusted Q values quantified the difference between the individual IV effects and the overall weighted average effect and their standard error. If Q_pval > .05, this indicates that heterogeneity is not significant, indicating that there is no statistically significant difference in the effects of the IVs, which was in line with the hypothesis of the IVW method. *P* values were used to determine the significance of this heterogeneity, which was used to assess whether the observed Q-value exceeded what would be expected under the assumption of no heterogeneity between IVs. Second, to further assess the robustness of the results, we conducted sensitivity analyses, including a leave-one-out analysis. This method detects whether a single SNP significantly influences the overall results by sequentially excluding each SNP, making it particularly suitable for causal analyses involving ≥2 IVs.[21] In addition, we used the Cochran Q test to assess heterogeneity in the IVW method and MR-Egger regression, in order to determine whether the causal effects estimated by each IV exhibit significant heterogeneity beyond the range of random expectation. In the absence of heterogeneity or when heterogeneity is not significant, a fixed-effects model was applied. In cases where heterogeneity is present, a random-effects model was used. The direction of the causal relationship was determined using the Steiger test.[22] All statistical analyses in this study were performed using the R programming language

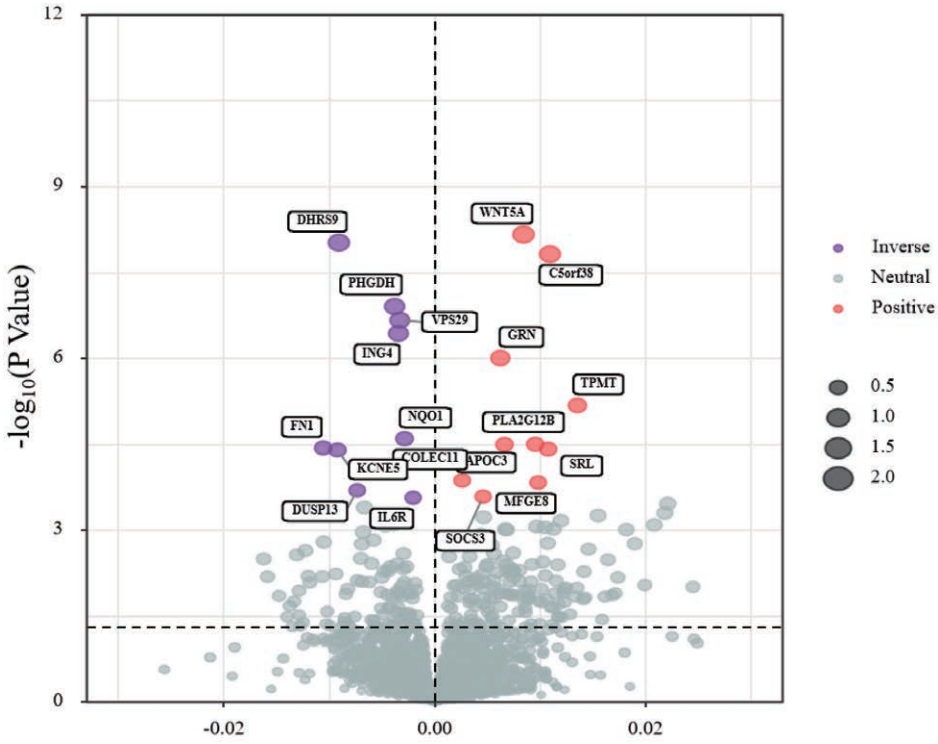

Figure 1. Volcano plot of Mendelian randomization (MR) results: the causal relationship between plasma proteins with potential causal effects and the risk of coronary atherosclerosis. FN1 = fibronectin 1.

and RStudio, with R version 4.3.1. The "TwoSampleMR" package, along with several basic R packages, was used for the analyses. A *P* value of <.05 was considered statistically significant. *P* < .05 was considered statistically significant.

2.4. Enrichment analysis

GO enrichment analysis enables the classification and comparison of genes and their RNA or protein products, providing a deeper understanding of their biological characteristics. GO enrichment analyses including biological process, molecular function, and cellular components can be used to identify genes that are thought to be closely related to the pathogenesis of coronary AS. The KEGG is a comprehensive dataset integrating genomic, disease, biological pathway, drug, and chemical information.[23] It is used to analyze the functions of genes and their products within networks, providing insights into signaling pathways. We performed detailed functional analyses of potential pathogenic proteins using the DAVID online tool (https://david.ncifcrf.gov/), with the enrichment significance threshold set at $P < .05$.[24]

2.5. PPI

The STRING database (http://string-db.org/) is a widely used resource for searching known and predicted protein-protein interactions, encompassing both direct functional interactions and indirect functional associations across various species. Using the STRING database, we integrated experimental data and PubMed abstracts to identify key regulatory proteins. The integration of genomic and proteomic data allows for the identification of critical protein networks associated with the disease, thereby revealing their roles in the disease process. We utilized the STRING database to construct an initial PPI network to investigate key genes associated with coronary AS and import the data into Cytoscape to construct subnetworks; STRING also incorporates information from other databases and utilizes bioinformatics methods for prediction.[25] Subsequently, Cytoscape is used to visualize molecular interactions and PPI networks, followed by a ranking analysis of key genes.

3. Results

3.1. Association between plasma proteins and the risk of coronary AS

In this study, we first conducted a 2-sample MR analysis to identify plasma proteins causally associated with coronary AS. Plasma proteins were used as exposure factors (with the number of IVs for each exposure ranging from 11 to 41). We calculated the *F*-statistics for the IVs of each identified AS-associated protein (with the *F*-values ranging from 31.28 to 3130.17); all *F*-values were >10, indicating that weak IVs were excluded, ensuring the validity of the selected IVs.

Through the preliminary MR analysis (primarily using the IVW method), we identified a total of 256 plasma proteins that have a causal relationship with the risk of AS ($P < .05$; Fig. 1). Twenty plasma proteins were identified as significantly associated with coronary AS; among them, 10 proteins may be associated with increased risk: WNT5A, C5orf38, GRN, TPMT, PLA2G12B, SRL, COLEC11, APOC3, MFGE8, and SOCS3. In contrast, 10 proteins may be associated with reduced risk: DHRS9, PHGDH, VPS29, ING4, NQO1, fibronectin 1 (FN1), KCNE5, IL6R, C1QTNF1, and DUSP13.

Afterward, heterogeneity was assessed to ensure robustness and validity by calculating Q_pval and *P* values through the IVW method. The results showed that after correction, a total of 16 plasma proteins, WNT5A, TPMT, COLEC11, APOC3, MFGE8, SOCS3, DHRS9, PHGDH, VPS29, ING4, NQO1, FN1, KCNE5, IL6R, DUSP13, and C1QTNF1, showed no heterogeneity ($P > .05$; Fig. 2). Since MR analyses may be influenced

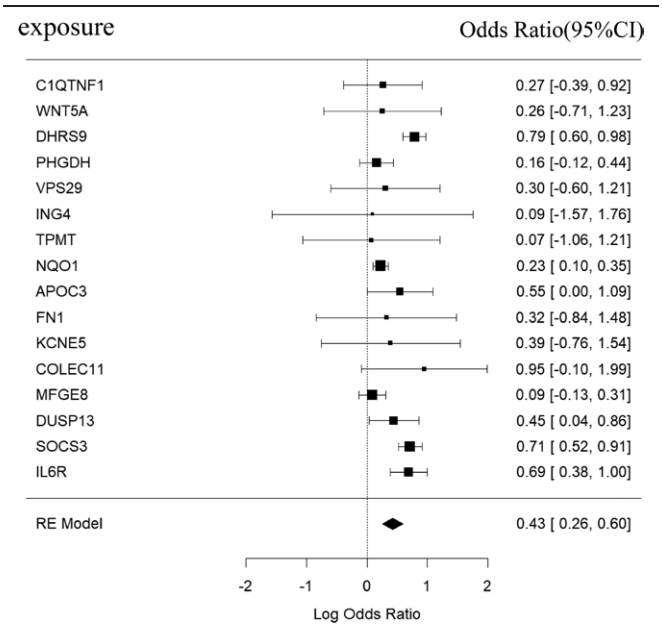

Figure 2. Forest plot of Mendelian randomization (MR) results. FN1 = fibronectin 1.

by the multiplicity of IVs, we conducted sensitivity analyses. The results of the leave-one-out method of analysis showed no significant change in the MR analysis after the exclusion of a single SNP, indicating the robustness of the results.

3.2. PPI network analysis construction and hub gene screening

In the preliminary MR analysis, we identified 256 plasma proteins with some level of association with coronary AS, of which 20 were significantly associated with coronary AS; to reveal the interactions between proteins potentially associated with coronary AS, a PPI network of the core genes was constructed using the STRING online tool, and the top ten highly expressed genes were visualized using Cytoscape software (Fig. 3A). The results showed that IL1B, FN1, EGF, CXCL12, SERPINE1, PLG, PLAU, THBS1, IGFBP3, and CRP were identified. The darker the color, the higher the score (Fig. 3B).

3.3. The results of gene enrichment

Through MR analysis, we ultimately identified 256 causal genes that may play significant roles in the development and progression of coronary AS. GO enrichment analysis is a common method used to reveal the relationships between genes and biological processes, while KEGG enrichment analysis is primarily used to explore the connections between genes and functional pathways. To explore the potential biological roles of these 256 genes, GO and KEGG analyses were performed using the DAVID database. GO enrichment analyses showed that biological process was most significant in signal transduction, apoptosis; cellular component mainly involves biological processes such as extracellular regions, extracellular fluids, and exosomes; and molecular function core genes mainly affect protein binding, protein exposure, and other protein-related biological functions. KEGG analysis indicated that the key genes mainly affect the following top 3 pathways: the PI3K-AKT signaling pathway, the cytokine-cytokine receptor interaction, and the MAPK-mediated signaling pathway. The PI3K-Akt signaling pathway is a crucial intracellular signaling pathway that plays a key role in regulating cell survival, proliferation, metabolism, and apoptosis. It has been demonstrated to have significant importance in cardiovascular diseases (Fig. 4).[26] Based on the MR analysis and PPI results, we identified FN1 as a key target gene. Scatter plots were generated accordingly, scatter plots showed a negative correlation of results, and FN1 was a protective factor for outcome (Fig. 5).

4. Discussion

In recent decades, significant progress has been made in both pharmacological and nonpharmacological treatments for atherosclerotic cardiovascular diseases, driven by advancements in medical technology. However, the global incidence and healthcare burden of the disease continue to rise, making it a major public health concern.[2] MR analyses commonly use genetic variants such as SNPs as IVs and have been extensively used in genetic epidemiology in recent years. MR studies provide a new perspective for understanding the causal effects of complex traits by identifying genetic variants associated with exposure variables and using these variants to infer causality. This study utilized various MR analysis methods based on large-scale data, high-statistical-power genomic analysis, and bioinformatics analysis. The study investigated the potential causal relationships between 1667 plasma proteins and the risk of coronary AS. Heterogeneity and robustness assessments were conducted based on the results to identify plasma proteins that may serve as potential therapeutic targets in the future. Our findings revealed that 20 plasma proteins are significantly associated with the risk of coronary AS. Through bioinformatics analysis, key target proteins were identified, which are of significant importance for predicting early disease risk of coronary AS, future diagnostics, and targeted drug therapy, highlighting their public health relevance. GO enrichment analysis revealed that these proteins are involved in various biological processes, primarily including protein-related biological activities, cellular components such as the extracellular region, and molecular functions such as receptor binding and receptor-ligand activity. KEGG analysis further highlighted the significance of the cytokine-cytokine receptor interaction pathway. PPI analysis revealed interconnections between these proteins, with the strongest interactions between IL1B, FN1, and EGF.

Previous studies have shown that coronary AS represents a fundamental physiological and pathological mechanism. Early arterial injury typically occurs in localized areas of the intima, followed by lipid deposition and intimal fibrous tissue proliferation; this process leads to gradual thickening of the intima, ultimately resulting in plaque formation. In our study, using a 2-sample MR approach, we identified a potential causal relationship between FN1 and AS. FN1 is a large glycoprotein located in the extracellular matrix, primarily secreted by fibroblasts, endothelial cells, and epithelial cells; the assembly of FN1 into insoluble multimers in the extracellular matrix is tightly regulated by cells; and it is involved in various cellular activities such as cell adhesion and migration. In studies investigating methods for isolating and culturing smooth muscle-derived fibroblasts from atherosclerotic plaques,[27] FN1, as a key extracellular matrix protein, was found to be stably expressed while maintaining proliferative capacity and transcriptional stability. FN1 encodes 2 distinct protein isoforms: plasma fibronectin and cellular fibronectin. These 2 protein isoforms function in the extracellular matrix and the circulatory system, respectively. Cellular fibronectin is enriched in the local matrix environment, serving as a crucial structural component of the extracellular matrix and playing a role in cell adhesion; plasma fibronectin is secreted by the liver into the circulatory system. Soubeyrand et al[28] conducted a 2-sample MR analysis using GWAS databases and introduced constructs encoding a truncated FN1 gene and a full-length plasma FN1 variant into various cell models. They found that higher levels of FN1 protein in the plasma were

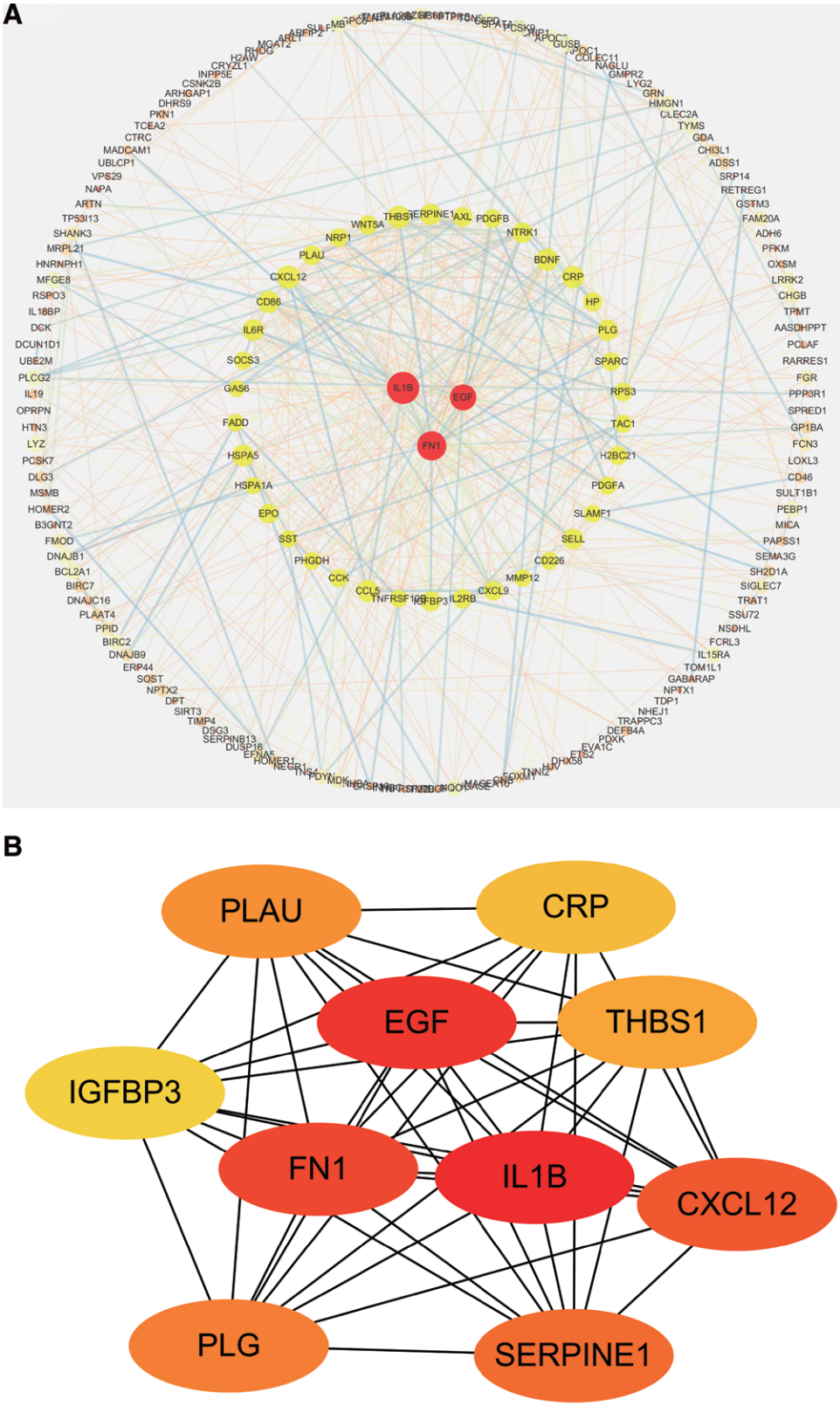

Figure 3. Results of the primary protein-protein interaction (PPI) network. (A) Primary PPI network analysis for coronary atherosclerosis. The color gradient represents the degree value, with red indicating a higher degree. (B) The core genes of the interaction network were obtained by the degrees algorithm. FN1 = fibronectin 1.

associated with a lower risk of CAD. FN1 expression levels increase during the progression of AS and are closely associated with the severity of the lesions. Dong et al[29] demonstrated that in endothelial cells, epsins promote the internalization of FN1, leading to its increased expression and degradation, which inhibits the TGF-β signaling pathway. This process enhances

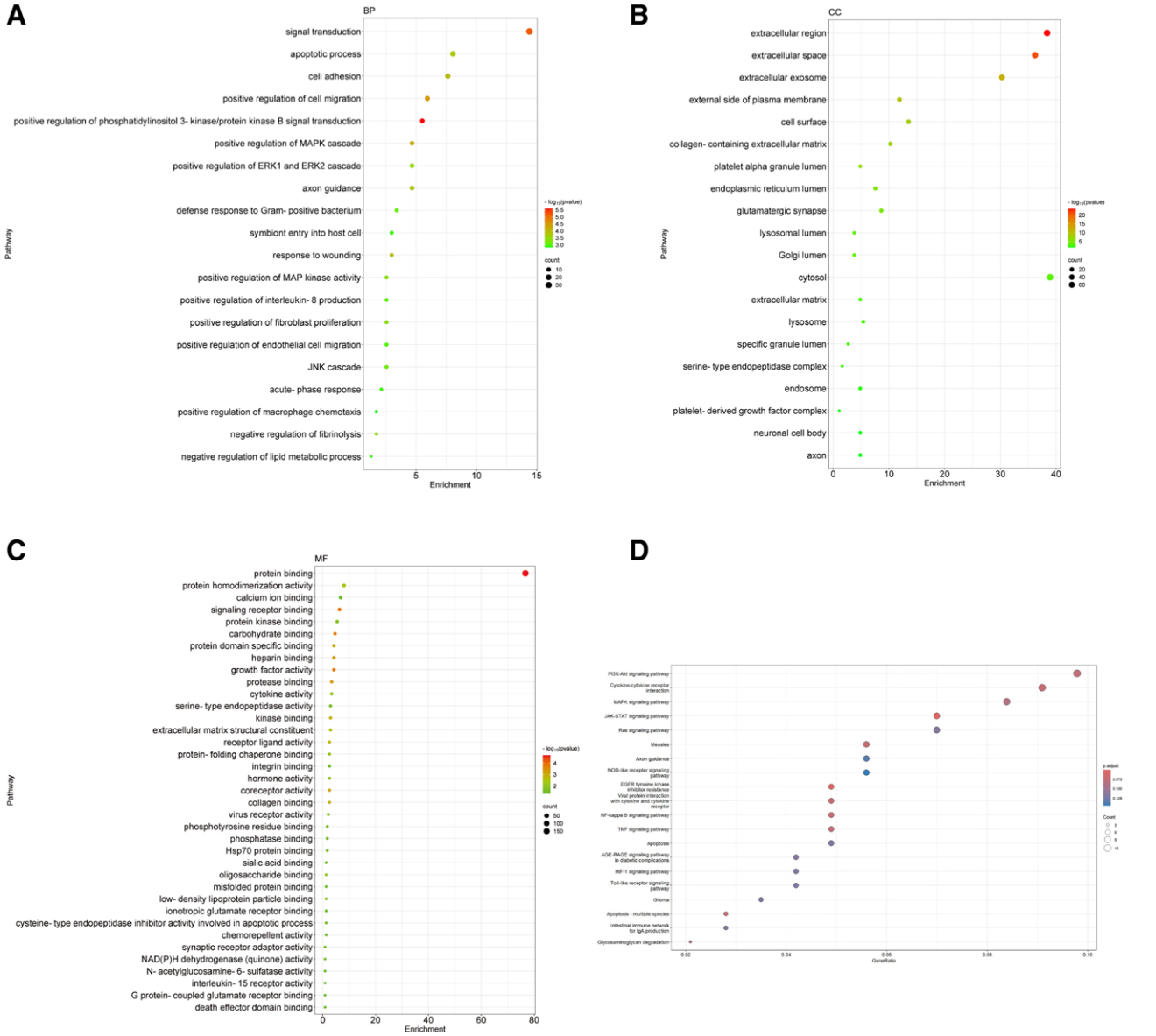

Figure 4. Enrichment pathway analysis of causal blood protein candidates. Gene Ontology (GO) enrichment results for 3 terms. (A) Biological processes (BPs). (B) Cellular components (CCs). (C) Molecular functions (MFs). (D) Pathway analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) candidate genes.

endothelial-to-mesenchymal transition during AS progression. Zhang et al[30] used gene analysis and proteomics studies in a carotid artery ligation mouse model to identify FN1 as one of the key genes involved in ligation-induced intimal hyperplasia. This finding enhances our understanding of AS and intimal hyperplasia. A recent study highlighted the potential of miRNA therapy in addressing dysregulated gene expression in AS.[31] Using single-cell RNA sequencing technology, the study identified FN1 as a key regulatory factor, opening new opportunities for the treatment of AS-related cardiovascular diseases. The inflammatory response is one of the key pathogenic mechanisms of AS; a study on the inflammatory gene expression profile of human SGBS adipocytes identified FN1 as a key molecule involved in the inflammatory response in AS.[32] The development and progression of AS involve multiple cell types, including macrophages, endothelial cells, and vascular smooth muscle cells; vascular smooth muscle cells are key players in the AS process, transitioning from a contractile to a synthetic phenotype. The increase in FN1 is associated with the proliferation and migration of vascular smooth muscle cells, contributing to plaque formation and progression. FN1 may also influence macrophage function and its distribution within plaques through interactions with receptors on the macrophage surface. In a study on mRNA profiles of whole blood samples from the site of coronary occlusion in patients with acute myocardial infarction, Muller et al[33] identified FN1 as one of the key genes that play a dominant role in the initial response to myocardial injury and its pathophysiological changes. Furthermore, FN1 is also associated with other vascular diseases caused by AS, such as hypertension and aortic calcification.[34–36]

Taken together, in the future, further research through large-scale cohort studies and advanced sequencing technologies will not only provide us with more comprehensive data and a deeper understanding of the underlying mechanisms but will also significantly advance the diagnostic and therapeutic approaches for coronary AS and the development of precision medicine at the genetic level.

Although previous studies have utilized MR to explore the relationships between various risk factors and the risk of coronary AS, our study is innovative in that it employs extensive GWAS and applies MR to specifically focus on the potential causal links between plasma proteins and coronary AS risk.

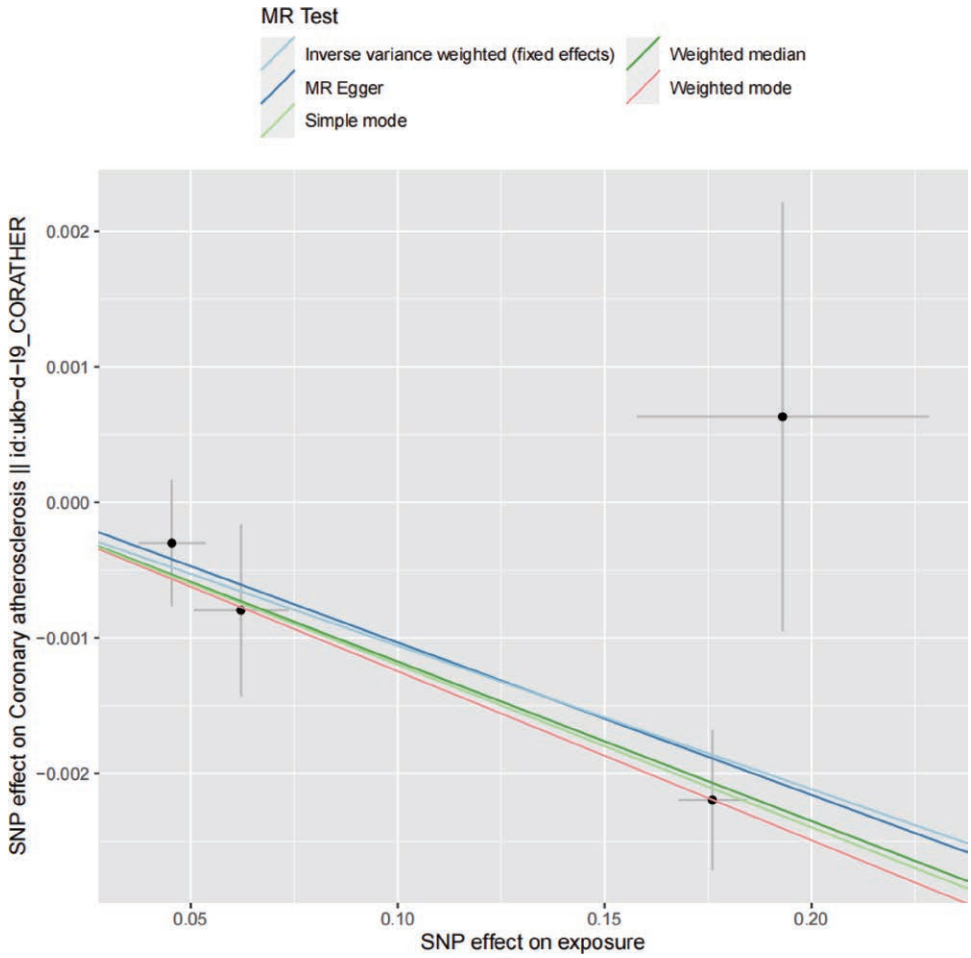

Figure 5. Mendelian randomization (MR) study results of hub genes. Scatter plot showing the causal effect of fibronectin 1 (FN1) on atherosclerosis (AS) risk. SNP = single-nucleotide polymorphism.

We performed variable selection based on the associations between specific proteins and disease, which reduced confounding bias and strengthened causal inferences, thereby significantly enhancing the genetic understanding of plasma proteins in relation to coronary AS and related diseases. In addition, we employed multiple MR methods for repeated analyses to ensure the objectivity, consistency, and robustness of the results. The screening of key plasma proteins is of great significance for the early identification of risk factors, risk stratification, and the development of precision prevention strategies for coronary AS. Finally, this study incorporated protein-protein interaction networks and pathway enrichment analyses, identifying several proteins associated with the mechanisms of coronary AS development and confirming their roles as potential therapeutic targets. This lays the foundation for understanding how key plasma proteins influence the pathways involved in the development and progression of coronary AS and for further exploring the role of the plasma proteome in coronary AS pathogenesis. Li et al[37] conducted a comprehensive integration of global genomic analyses and molecular trait data through a probabilistic Mendelian randomization approach, identifying 30 candidate proteins that are causally linked to CAD. This work also provides valuable insights for future research on CAD and the development of drug targets. In contrast, our study employed different methodologies and databases, placing greater emphasis on elucidating the causal relationship between plasma proteins and coronary AS. We highlight FN1 as a crucial protective factor involved in the PI3K-AKT signaling pathway. Notably, FN1 was included in Li's results, further enhancing the credibility of our findings. Furthermore, this study utilizes a classic MR framework characterized by detailed data transparency and robust causal inference methodologies. It offers more precise guidance for disease intervention and drug development while possessing deeper clinical significance.

However, our study still has certain limitations. Because this study is based solely on European databases, it lacks data from other ethnicities, which may lead to heterogeneity in genotype-phenotype associations. In addition, this study did not include data on plasma protein levels from other tissues. Therefore, further research using more diverse GWAS databases and more in-depth experimental approaches is needed to validate the findings; conducting additional long-term cohort studies or clinical trials with extended follow-up periods could more effectively control potential biases and provide clearer evidence for causal relationships. Therefore, to further deepen the findings of this study, prospective studies could be conducted to validate the association between the levels of key genes and the risk of coronary AS; future research should incorporate both in vitro and in vivo models to explore the underlying cellular and biological mechanisms, construct a protein biomarker network system that integrates genotype, proteomic, and clinical data, enhance the sensitivity and specificity of coronary AS prediction, and improve the therapeutic potential of core targets.[38,39]

5. Conclusion

This study, through MR analysis, revealed causal relationships between several plasma proteins and coronary AS, deepening our understanding of the pathogenesis and therapeutic approaches

of coronary AS. Further analysis identified 20 potential therapeutic targets, with FN1 being identified as a key target, showing significant biological effects. It provides new evidence and insights for exploring potential therapeutic targets. These findings not only provide new approaches for clinical prevention and treatment but also lay the foundation for developing more effective therapeutic strategies in the future. Further research should focus on functional validation and mechanistic studies of plasma proteins to drive innovation and optimization of therapeutic strategies for coronary AS.

Author contributions

Conceptualization: Henan Pan, Yaran Gao, Wentao Yao.
Data curation: Henan Pan, Yaran Gao, Wentao Yao.
Writing – original draft: Henan Pan, Yaran Gao.
Writing – review & editing: Henan Pan, Wentao Yao.
Software: Zongkai Wu, Ge Feng.
Supervision: Zongkai Wu, Ge Feng.
Validation: Yaran Gao.
Formal analysis: Hebo Wang.
Investigation: Hebo Wang.
Methodology: Hebo Wang.
Project administration: Hebo Wang.
Resources: Hebo Wang.

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